# ***PHENOL DEGRADATION USING MICROORGANISM***

Thesis submitted by

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In partial fulfillment for the award of the degree of

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Under the guidance of

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# CERTIFICATE

This is to certify that the thesis entitled **"Phenol degradation using microorganisms"** submitted by Jigar Jayant Sakariya (111ch0364) in partial fulfillment of the requirements for the award of BACHELOR OF TECHNOLOGY Degree in Chemical Engineering at the National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by them under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any degree or diploma

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## ***ABSTRACT***

Pollutants have adverse effect on environment and public health. From all the above pollutants given phenol is most toxic organic compound present in environment as pollutant. EPA (1998) has declared that $11^{th}$ out of 126 toxic chemicals is phenol. All the processes available for phenol degradation are not economically viable hence biodegradation is recommended since it is pollution free and also economically viable. In the below study three microorganism isolated from soil were compared for phenol degradation. The comparison was done on basis of percentage of phenol degraded, optimum temperature, optimum media $P_H$ and the inoculum age and volume. From the comparative study the best microorganism strain was found out to be $PR_3$. This microorganism was found to be degrading maximum phenol of 76% and at temp of about $30^oC$ and pH of around 7 with inoculum age between 20-24 hours. After characterisation this microorganism was found to be *staphylococcus lentus*

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# 1. INTRODUCTION

Water covering the large earth surface is also a valuable asset for human life. Water covers around 69.839% of the earth surface area. If water goes to extinct the life on earth will stop. This includes the fuel industries such as petroleum and petroleum products industries, paint industries, dyes industries etc. Among these pollutants one of the harmful pollutant is phenol which has been declared by EPA (1998) to be11$^{th}$ out of 126 toxic chemicals. Also phenol is toxic in nature and causes various diseases. Phenol and phenol vapor are corrosive to eyes, skin, and respiratory tract. Continuous or longer skin contact with phenol and phenolic vapour may cause dermatitis and second or third-degree burns. Inhalation of its vapor (more than 0.45ppm by EPA) can cause disease lungedema. This have dangerous effect on human health. The effect of this is seen on the nervous system this also has effect on heart. Phenols also form the dangerous and most corrosive phenoxylic radicals. Kidneys and also livers can have many long term effects. Hence removal of phenol is essential from the industrial effluents before letting them as wastes.

There are several ways to remove phenol from the industrial effluent or from the phenol contaminated water this methods are other than the biodegradation method for phenol. Phenol has various effects on human being both short term and long term. Various methods use for phenol separation are electro coagulation, extraction, adsorption and ion exchange, advanced oxidation process, electro-fenton method, membrane separation, photodecomposition etc. This all methods are used for effective phenol separation. Adsorption is used extensively to remove phenol from effluent waste. It was determined that the particle size and flow rate are the cause for sorption of phenol, and if we increase flow rate and particle size the particle size and break time point also decreases. If adsorbent is used 89-97% removal of phenol can be guaranteed. Other method is electro-coagulation it is basically based on the electro chemical removal of compounds. This method has been adopted by many petroleum industries to remove phenol. But these all processes are very expensive and difficult to handle. Biological degradation of phenol by using microorganism is such a technique to remove phenol from phenol contaminated water. This process unlike the above ones is toxic free process it has no by products and more importantly it is cost effective process. This process can be handled easily with less equipment and is economically viable.

There are several advantages of biodegradation over all the above methods which were discussed for removal of phenol from the toxic effluent. First advantage of microbial degradation is the pathway that the microorganisms follow which is capable of breakage of these toxic compounds into the simpler and nontoxic compounds. These methods has no by products and has eliminated the problem of handling waste from the degradation process. Also this method does not involve usage of costly commercial equipment such as the distillation column or high temperature reactors hence it is economical for both small scale and large scale operations. This method also eliminates the cost of the costly chemicals used for oxidation reaction and risk factor of handling those reactive oxidizing agent. At last this process can be done at room temperatures as microorganisms growing conditions are at room temperature itself. Hence biodegradation is used for degrading phenol.

Extensive research work has been done in field of biodegradation and several microorganisms are found which have the capability to degrade phenol. Microorganisms like bacteria, fungi and actionmytes degrade phenol to a large extent. Bacterial species include *Pseudomonas sp*, *Bacillus sp*, *Achromobacte*r *sp Acinetobacte*r *sp* etc. *Fusarium sp*, C*orious versicolo*r, *Phanerocheate chrysosporium*, *Streptomyces sp*, *Ralstonia sp* etc are the researched fungi in the phenol removal. Hence in our study we will use the biodegradation method to degrade phenol by microorganisms collected from the contaminated sight. And optimum conditions will be compared for these microorganisms and the best one will be found and characterised.

# 2. LITERATURE SURVEY

## 2.1 PHENOL:

Phenol also known as organic acid, any of a family of organic compounds characterized by a hydroxyl (−OH) group attached to a carbon atom that is part of an aromatic ring. Besides serving as the generic name for the entire family, the term *phenol* is also the specific name for its simplest member, mono-hydroxy benzene ($C_6H_5OH$), also known as benzenol or carbolic acid. Phenol was first extracted from coal tar, but today is produced on a large scale (about 7 billion kg/year) from petroleum. It is an important industrial commodity as a precursor to many materials and useful compounds. Its major uses involve its conversion to plastics or related materials. Phenol and its chemical derivatives are keys for building polycarbonates, Bakelite, nylons, detergents, and numerous pharmaceutical drugs.

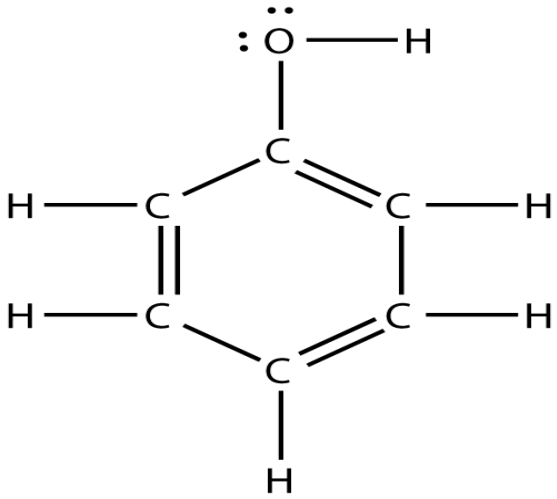

Fig 1.1 phenol structure

Phenols are similar to alcohols but form stronger hydrogen bonds. Thus, they are more soluble in water than are alcohols and have higher boiling points. Phenols occur either as colourless liquids or white solids at room temperature and may be highly toxic and caustic. Phenols are common in nature; examples include tyrosine, one of the standard amino acids found in most proteins; epinephrine (adrenaline), a stimulant hormone produced by the adrenal medulla; serotonin, a neurotransmitter in the brain; andurushiol, an irritant secreted by poison ivy to prevent animals from eating its leaves. Many of the more complex phenols used as flavourings and aromas are obtained from essential oils of plants. For

example, vanillin, the principal flavouring in vanilla, is isolated from vanilla beans, and methyl salicylate, which has a characteristic minty taste and odour, is isolated from wintergreen. Other phenols obtained from plants include thymol, isolated from thyme, and eugenol, isolated from cloves.

### ***2.1.1 PROPERTIES OF PHENOL*:**

| **PROPERTIES** | **PHENOL** |
|---|---|
| Chemical formulae of compound | $C_6H_5OH$ |
| Molecular weight of phenol | 94 |
| Boiling point($^oF$) | 359.6(182$^oC$) |
| Melting point($^oF$) | 104.9(40.2$^oC$) |
| Solubility in water(g/100ml) | 8.3(at 20$^oC$) |
| Density($g/cm^3$) | 1.069 |
| Appearance | Transparent crystalline solid |
| Odor | Sweet and tarry |
| Acidity($pK_a$) | 9.95(in water) |
| Flash point($^oC$) | 87 |

Table 2.1 physical properties of phenol.

### ***2.1.2 PHENOL USES:***

Phenol is a versatile precursor to a large collection of drugs, most notably aspirin but also many herbicide and pharmaceutical drugs. Phenol is also used as an oral analgesic in products such as Chloraseptic or other brand name and generic equivalents, commonly used to temporarily treat pharyngitis. The primary use of phenol is in the production of phenolic resins, which are used in the plywood, construction, automotive, and appliance industries. Phenol is also used in the production of caprolactam and bisphenol A, which are intermediates in the manufacture of nylon and epoxy resins, respectively. Other uses of phenol include as a slimicide, as a disinfectant, and in medicinal products such as ear and nose drops, throat lozenges, and mouthwashes.

Phenol is also used for permanent treatment of ingrown toe and finger nails, a procedure known as a chemical matrixectomy. The procedure was first described by Otto Boll in 1945. Since that time it has become the chemical of choice for chemical matrixectomies performed by podiatrists. Phenol derivatives are also used in the preparation of cosmetics including sunscreens, hair colourings. Concentrated phenol liquids are commonly used in the surgical treatment of ingrown toenails to prevent a section of the toenail from growing back. This process is called phenolization.

### ***2.1.3 TOXICITY OF PHENOL:***

Phenol is extremely toxic compound. If not handled carefully it can lead to several physical damages to human body. Phenol and its vapors are corrosive to the eyes, the skin, and the respiratory tract. Repeated or prolonged skin contact with phenol may cause dermatitis, or even second and third-degree burns. Inhalation of phenol vapor (more than 0.45ppm by EPA) may cause lungedema. The substance may cause harmful effects on the central nervous system and heart, resulting in dysrhythmia, seizures, and coma. Besides its hydrophobic effects, another mechanism for the toxicity of phenol may be the formation of phenoxyl radicals. The kidneys may be affected as well. Long-term or repeated exposure of the substance may have harmful effects on the liver and kidneys. There is no evidence that phenol causes cancer in humans.

### *2.1.4 AMOUNT OF PHENOL RELEASED IN ENVIORNMENT:*

Different industries releases different amount of phenol in the atmosphere. Some of the industrial survey is given in form of table below.

| **NAMES OF INDUSTRIES** | **CONC. OF PHENOL RELEASED**(mg/L) |
|---|---|
| BILT paper industries | 1400-1500 |
| Barauni petroleum refinery | 450-550 |
| Paradeep petrochemicals | 1000-1160 |
| Rourkela steel plant | Up to 3500(for coking process) |

Table 2.2 Amount of phenol released by different industries.

### *2.1.5 DIFFERENT METHODS FOR PHENOL REMOVAL:*

There are several ways to remove phenol from the industrial effluent or from the phenol contaminated water this methods are other than the biodegradation method for phenol. Phenol has various effects on human being both short term and long term. Various methods use for phenol separation are electro coagulation, extraction, adsorption and ion exchange, advanced oxidation process, electro-fenton method, membrane separation, photodecomposition etc. This all methods are used for effective phenol separation.

#### *2.1.5.1 ADSORPTION AND ION EXCAHNGE*:

Experiments were conducted to examine the liquid phase adsorption of phenol from contaminated eater using the silica gel, activated carbon and alumina by Roostaei and Tezel [26]. Caetano et.al. [27] also carried out the same experiment of adsorption of phenol on the polymeric resins. Adsorption equilibrium capacities and kinetics analysis were carried out and it has found to be a promising method for phenol removal. They found that that the sorption of phenol is dependent on both the flow rate and the particle size of the adsorbent, and that the breakpoint time and phenol removal yield decrease with increasing flow rate and particle size. Phenol removal ranging from 88 % to 95 % was observed using various adsorbent. Adsorption seems to be possibly most widely studied operation for phenol.

### *2.1.5.2 ELECTRO COAGULATION:*

It is basically based on the electro chemical removal of compounds. This method has been adopted by many petroleum industries to remove phenol. Abdelvahab et.al. have carried out the research on electrochemical removal of phenol from oil refinery waste . In this process of electrochemical separation of phenol they used a tank with horizontal aluminium rods and the aluminium screen acting as anode. They studied the phenol removal with respect to various parameters such as pH, operating time, current density, initial phenol concentration and addition of NaCl. According to them removal of phenol during electro coagulation was due to combined effect of sweep coagulation and adsorption. The research studies showed that the 97% of separation was obtained after 2 hours of time at the operation $p_H$ of 7.

### *2.1.5.3 EXTRACTION:*

Extraction is the most common process for separation of many compounds from each other. Extraction has been used in various industrial applications. Extraction is the process in which solvent is used to dissolve phenol from the effluent and then this solvent is separated by the distillation process. This process is very common but the important factor here is the selection of the solvent by the help of which we can separate the phenol. The solvent should be selected in such a way that it could absorb the maximum amount of the phenol and separation of phenol from the solvent should be economic and more viable. The solvent which is selected for the separation or the extraction process should be non-toxic in nature. There are many organic compounds which can be used for phenol as the solvents. 1-hexanol, 1-heptanol and 1 octanol can be used as solvent for phenol removal

## *2.2 DRAWBACKS OF ALL THE ABOVE METHODS:*

All the methods discuss above are either the physical process or chemical process or physico-chemical process. This all the procedure involve lots of apparatus and handling of the chemical compounds. The apparatus involved in this type of the process deals with the high input of energy even for the mere separation of phenols. These processes are also not economically viable and have the release of all toxic by-products and toxic wastage in environment. By using these methods it is like trading one toxic element at the expense of other. The ion-exchange resins used in the adsorption process are too costly for the process to get fit into the economic agenda.

The studies in various field of different methods for phenol degradation is appreciative, but among the above mentioned and discussed process none of the above process can be performed on the large scale. Moreover all the above method are $p_H$ sensitive and hence the maintenance of the accurate $p_H$ becomes the most difficult part to be handled where the large scale processes have been in use. Also the phenol which is being separated from all the above process is not processed further. The solid crystals of phenol are obtained and these are in form of waste and again needs a safe disposal. For example consider the extraction process. After separating phenol from the solvent its again a solid waste which needs to be disposed of or been recycled to industries which use phenol for the production of various products. The various product industries producing cosmetics, antiseptics, etc can import this waste phenol from the separation units.

In case of the adsorption process if we use activated carbon to remove or adsorb phenol from the effluent waste the recovery of activated carbon is very difficult, costly and most energetic process. The oxidation process used for phenol separation is the efficient process which can remove 99.99% of phenol by oxidation process. This process is based mostly on chemical reactions. There are several by products which are produced by this method which are of no use. This method in fact produces toxic waste by processing the toxic chemical. Also the cost of chemicals used here is so high that some times for a large scale removal of phenol is not possible due to economic issues. Many oxidizing reagents used in this process for example hydrogen peroxide, causes incomplete oxidation for many other organic compounds present in the effluent water which are toxic in nature.

Hence it has been extremely important to find a method that would be free of all above drawbacks and which would be efficient enough to degrade phenol at comparable level. Biological degradation of phenol by using microorganism is such a technique to remove phenol from phenol contaminated water. This process unlike the above ones is toxic free process it has no by products and more importantly it is cost effective process. This process can be handled easily with less equipment and is economically viable.

### *2.3 BIODEGRADATION AND ITS ADVANTAGES:*

Biodegradation is the process which uses microorganisms to breakdown the toxic chemicals or pollutants to simple and nontoxic compounds by microbial metabolism. Here the microorganisms grow on these toxic compounds as their food. They breakdown this toxic compounds into the simpler and harmless compounds. The microorganisms for these transactions follow several pathways which will be discussed later. Alexander in 1995 have explained that microorganism have the capability of degrading any compound. This capability of microorganism is called microbial infallibility. But a single microorganism cannot degrade or survive in all kind of conditions. Specific microorganism can only degrade a specific compound or in other words we can say that specific microorganism take only specific compounds as their food.

There are several advantages of biodegradation over all the above methods which were discussed for removal of phenol from the toxic effluent. First advantage of microbial degradation is the pathway that the microorganisms follow which is capable of breakage of these toxic compounds into the simpler and nontoxic compounds. These methods has no by products and has eliminated the problem of handling waste from the degradation process. Also this method does not involve usage of costly commercial equipment such as the distillation column or high temperature reactors hence it is economical for both small scale and large scale operations. This method also eliminates the cost of the costly chemicals used for oxidation reaction and risk factor of handling those reactive oxidizing agent. At last this process can be done at room temperatures as microorganisms growing conditions are at room temperature itself. The only problem regarding the use of this method is that it needs to be optimized and microorganism or biomass used for this method should be checked because if the dead mass increases in the reacting vessel it can clog the flow pattern of the input and

output streams. List of various microorganisms used for this purpose are listed in the table below:

| MICROORGANISMS | ITS USAGE |
|---|---|
| ***M.rouxii(fungi)*** | Oil removal from water |
| ***Pseudomonas putida*** | Removal of phenol from water |
| ***Absidia coerulea*** | Removal of hydrocarbon |
| ***Candida tropicalis*** | Waste water treatment |
| ***Sphingomonas chlorophenolica*** | Pentachlorophenol |

Table 2.3 Applications of microorganisms in various fields.

### *2.4 RESEARCH PAPER STUDY:*

Basically phenol is not microbial friendly in nature. Its antimicrobial activity is well known and hence it is very difficult to find the microorganisms which can degrade phenol and its derivatives. But despite of all this difficulties researchers have been successful in degrading phenol effectively by using the microbial culture. Researchers have also found out microorganisms which are susceptible to this compound and its derivative. But still there are microorganisms which can degrade and resist the toxicity of phenol. There are several microorganisms which are found capable of degrading phenol Degradation of phenol occurs as a result of the activity of a large number of microorganisms including bacteria, fungi and actinomycetes.

Many other microorganisms which are found to be degrading phenol from various species are been mentioned here. *Bacillus stearothermophilus* (Gurujeyalakshmi and Oriel, 1988), *Pseudomonas putida* (Allsop et al, 1993), Immaculate colonies of Pseudomonas putida (ATCC 17484) were developed in constant culture on phenol at diluting rates of 0.074–0.085 $h^{-1}$ and subjected to step increments in phenol concentration. Three unmistakable examples of

element reaction were gotten relying upon the span of the step change utilized: low level, moderate level, or abnormal state. Amid low level reactions no collections of phenol or non-phenol, non-glucose-disintegrated natural carbon, DOC(NGP), were watched. Moderate level reactions were described by the transient amassing of DOC(NGP) with a critical postponement before phenol spillage. These results were consistent with intermediate metabolite production during phenol step tests coupled with substrate-inhibited phenol uptake and suggested that traditional kinetic models based on the Haldane equation may be inadequate for describing the dynamics of phenol degrading systems.

Numerous studies on biodegradation of phenol originate from microscopic organisms. The sort Pseudomonas is generally sought the debasement of phenolic mixtures. These microbes are known for their capacity to grow on numerous compounds. Phenol biodegradation studies with the bacterial species have brought about drawing out the conceivable component furthermore the chemical included in the process. The effectiveness of the phenol degradation could be further improved by the procedure of cell immobilization (Annadurai et al., 2000a, b). Phenol and other phenolic mixtures are regular constituents of numerous modern effluents. When a suitable smaller scale microorganism based methodology is produced for the successful debasement of phenol these phenolic effluents can be securely treated and arranged (Borghei and Hosseini, 2004).

Candida tropicalis from the gushing of the Exxon Mobile Oil Refinery waste water treatment was researched for phenol degradation utilizing group and bolstered bunch maturation under aerobic condition (Mohd Tuah, 2006). Microbial degradation of phenol and some of its alkyl subsidiaries (p-cresol, 4-n-propyl phenol, 4-i –propyl phenol, 4-n-butyl phenol, 4-sec-butyl phenol, 4-t-butyl phenol and 4-t-octyl phenol) were analyzed under both high-impact and anaecrobic conditions in seven Japanese paddy soil tests (Atsushi et al., 2006). The rate of biodegradation of phenol by Klebsiella oxytoca strain was examined. It was discovered that K. oxytoca degraded phenol at raised fixation where 75% of introductory phenol of concentration 100 ppm was degraded inside 72 h (Shawabkeh et al., 2007). Phenol was degraded by Actinobacillus species (Khleifat and Khaled, 2007). They discovered that pH 7, the temperature of 35 to 37°C, and the rate of 150 rpm were the ideal conditions for accomplishing the higher rate of phenol degradation.

Many others have also researched in this field *Pseudomonas putida* Loh and Wang (1998), *Pseudomonas flurorescens* Torres et al. (1998), *Pseudomonas putida* Mordocco et al. (1999), *Pseudomonas sp* Gonzalez et al. (2001), *Pseudomonas putida* Loh and Jun (2001), *Pseudomonas putida* Petruschka et al. (2001), *Pseudomonas putida* Kargi and Eker (2005) and *Pseudomonas sp* Prpich and Douglis (2005)

*Lentinus bisporous* (Okeke et al, 1993), The development of four strains of the shiitake mushroom Lentinus edodes in strong substrate maturation in manufactured oak sawdust logs was examined over a 14-week period. All out extracellular phenol oxidation and solvent protein were checked and biomass evaluated as the ergosterol substance of the matured sawdust. It was watched that two of the strains had a comparative example of phenol oxidase action with two cycles with maxima at 2 and 8 weeks of mycelial development before fruiting. With the other two strains there was a greatest at week 4. For every strain, phenol oxidase action expanded with the cool stun used to actuate fruiting. Phenol oxidase movement was not discovered to be associated with either dissolvable protein or aggregate contagious biomass in the aged sawdust, which were related for every strain. Evaluation of biomass from submerged fluid culture on the premise of dry weight and ergosterol substance demonstrated that the strains fell into the same two gatherings as for the ergosterol to biomass proportion, which was extraordinarily lower than that for a strain of L. lepideus.

*Acinetobacter johnsonii* (Hoyle et al, 1995), In contaminated soil or ground water, inorganic supplements, for example, nitrogen may be constraining, so that Monod energy for carbon constraint may not depict microbial development and contaminant biodegradation rates. To test this speculation we measured $^{14}CO_2$ advanced by an immaculate culture of Acinetobacter johnsonii degrading 120 µg $^{14}$C-phenol every ml in soaked sand with molar carbon : nitrogen (CN) proportions running from 1.5 to 560. We fit energy models to the information utilizing non-straight slightest squares relapse. Phenol vanishing and biomass created were likewise measured at CN1.5 and CN560. After a 5 to 10 hour slack period, the majority of the $^{14}CO_2$ advancement bends at all CN proportions showed a sigmoidal shape, recommending that the microbial biomass developed. As CN proportion expanded, the

introductory rate of $^{14}CO_2$ development diminished. Cell development and phenol utilization happened at both CN1.5 and CN560, and demonstrated the same patterns as the $^{14}CO_2$ information. An energy model accepting biomass production restricted by a solitary substrate best fit the $^{14}CO_2$ advancement information for CN1.5. At intermediate to high CN ratios, the data were best fit by a model originally formulated to describe no-growth metabolism of one substrate coupled with microbial growth on a second substrate. We suggest that this dual-substrate model describes linear growth on phenol while nitrogen is available and first-order metabolism of phenol without growth after nitrogen is depleted.

From S. Sivasubramanian, S.K.R. Namasivayam (Journal of Environmental Chemical Engineering 3 (2015) 243–252), A microbial consortium consisting of five phenol-degrading strains was developed. The consortium could degrade 99% of 1000 mg/L phenol after 72 h. Characterization of the members revealed that consortium consisted of *Candida tropicalis, Aspergillus fumigatus, Candida albicans, Candida haemulonis* and *Streptomyces alboflavus.* Performance of consortium was accessed by specific growth rate and yield coefficient. The consortium exhibited activity of phenol hydroxylase and catechol 1,2-dioxygenase suggesting that members in consortium followed ortho pathway for phenol degradation. Response surface methodology has been adopted to evaluate the effect of different parameter such as initial phenol concentration temperature, pH and time on phenol degradation. The maximum percentage of phenol degradation is observed at optimized value of process variables such as initial concentration 1000 mg/L, temperature 35$^{o}$C, pH 7 and incubation time of 96 h.

*Azoarcus* species (Paula.M and L.Young et. al, 1997), Phenol is a man-made as well as a naturally occurring aromatic compound and an important intermediate in the biodegradation of natural and industrial aromatic compounds. Whereas many microorganisms that are capable of aerobic phenol degradation have been isolated, only a few phenol-degrading anaerobic organisms have been described to date. In this study, three novel nitrate-reducing microorganisms that are capable of using phenol as a sole source of carbon were isolated and characterized. Phenol-degrading denitrifying pure cultures were obtained by enrichment culture from anaerobic sediments obtained from three different geographic locations, the East River in New York, N.Y., a Florida orange grove, and a rain forest in Costa Rica. The three strains were shown to be different from each other based on physiologic and metabolic

properties. Even though analysis of membrane fatty acids did not result in identification of the organisms, the fatty acid profiles were found to be similar to those of *Azoarcus* species. Sequence analysis of 16S ribosomal DNA also indicated that the phenol-degrading isolates were closely related to members of the genus *Azoarcus*. The results of this study add three new members to the genus *Azoarcus*, which previously comprised only nitrogen-fixing species associated with plant roots and denitrifying toluene degraders.

*Ochromonas danica* (Semple and Cain, 1997), In this study, the catabolic adaptability of the eukaryotic alga Ochromonas danica (CCAP 933/2B) to degrade mixtures of, instead of individual, phenols was examined. O. danica, after development on phenol, had the capacity metabolize 2,5-xylenols, 2,6-xylenols and 3,5-xylenols just in the vicinity of phenol in the incubation medium. Once the phenol had been depleted (2–3 h) from a phenolic mixture in the incubated media, there was a critical diminishment in the rates of xylenol isomer evacuation and none of these xylenols (250 μM) was totally used in 6 h. 3,4-Xylenol, in any case, was totally expelled from the development medium in 3 h, despite the fact that all the phenol had vanished in 2 h. O. danica can metabolize mixtures of phenols known to represent an ecological risk. In spite of the fact that it is at present hazy exactly how broadly past the immediate ring cleavage products the all the more very substituted alkylphenols can be degraded, it is obvious that some green growth may add to the turnover of carbon connected with mixtures of those phenolic mixes found in waste water.

*Phormidium valderianum* (Shashirekha et al, 1997), In this new innovation which stresses the detoxification and devastation of the toxins by acclimatized microorganisms, *Cyanobacteria* are in an a greater number of worthwhile position than heterotrophic microscopic organisms due to their trophic freedom for nitrogen and also carbon. Phenol, the dangerous constituent of a few modern effluents, was discovered to be successfully uprooted and debased by the marine *cyanobacterium Phormidium valderianum* BDU 30501. The organic entity had the capacity endure and develop at a phenol amassing of 50 mg L−1 and uproot 38 mg L−1 inside a maintenance time of 7 days. The evacuation and corruption were affirmed by changes in the bright retention spectra in the way of life filtrate, colorimetric estimation of remaining phenol and measuring the intracellular action of the inducible polyphenol oxidase and laccase proteins. This opens up the likelihood of treating a mixed bag

of phenol-containing modern effluents utilizing this microorganism. But this process was very time consuming and needed to be optimised using the supply of neutrients in additional to industrial effluents as the only food available for microorganisms.

*Rhizoctonia praticola* (Bollag et al, 1988), The capacity of a polyphenoloxidase, the laccase of the parasite Rhizoctonia praticola, to detoxify phenolic toxins was analyzed. The development of the growth could be restrained by phenolic mixes, and the compelling focus was reliant on the substituents of the phenol. A poisonous measure of a phenolic compound was added to a parasitic development medium in the vicinity or non attendance of a regularly happening phenol, and 50% of the imitates likewise got laccase. The medium was then immunized with R. praticola, and the levels of phenols in the medium were observed by elite fluid chromatography investigation. The expansion of the laccase switched the inhibitory impact of 2,6-xylenol, 4-chloro-2-methylphenol, and p-cresol. Different mixes, e.g., o-cresol and 2,4-dichlorophenol, were detoxified just when laccase was utilized as a part of conjunction with a characteristic phenol, for example, syringic corrosive. The lethality of p-chlorophenol and 2,4,5-trichlorophenol couldn't be overcome by any augmentations. The capacity of the laccase to modify the poisonous quality of the phenols seemed, by all accounts, to be identified with the limit of the catalyst to lessening the levels of the guardian compound by change or cross-coupling with another phenol.

*Trametes trogii* (Garzillo et al, 1998), The white-decay growth Trametes trogii discharges a fundamental laccase demonstrating an atomic mass of 70 kDa, acidic isoelectric point and N-terminal succession homologous to that of a few phenol oxidases. The cleaned compound oxidizes various phenolic and non-phenolic mixes; obstinate particles may be changed over into substrates by presenting, in the right position, o- or p- situating ring-enacting gatherings. It is perceived that cellulase creation in most cellulolytic organic entities is actuated by the vicinity in culture media of either low-sub-atomic mass cellulose subordinates or cellulose-containing materials (Coughlan 1985; Uzcatequi et al. 1991; Garzillo et al. 1994). This wonder was likewise seen amid the development of T. trogii mycelium and the levels of discharged cellulases, both regarding units/ml and units/mg protein, expanded continually when the wheat straw was expanded from 1% to 6% in the media.

*Coriolus versicolor* (Kadhim et al, 1999), colony filtrate removed from Coriolus versicolor developed on wheat grain supplemented with yeast concentrate was utilized to uproot a scope of phenolic mixes. The evacuation was because of the vicinity of laccase. Mn-subordinate peroxidase was additionally show however was unequivocally inactivated by the phenolic mixes explored, recommending it may not be included in the evacuation process. Laccase was likewise inactivated to some degree. The impact of the position of substitution of chlorine on the benzene ring and its impact on the evacuation was analyzed. Phenolic mixes with chlorine substitutions in ortho and para positions were effectively assaulted by the protein dissimilar to mixes with substitutions in the meta position. Moreover, the impact of arrangement oxygen fixation on the evacuation of phenolic mixes was examined. An increment in broke up oxygen focus brought about expanded evacuation effectiveness.

*Ralstonia eutropha* (Leonard et al, 1999 a,b), The impact of phenol concentration on development and biodegradative limit of Ralstonia eutropha in regards to phenol was inspected. kinetic analysis demonstrated that phenol had a solid inhibitory impact on phenol metabolism and development rate, in spite of the fact that biomass yields stayed consistent, showing that this phenomena was not created by expanded support prerequisites. Estimations of particular protein activities included particularly in the catabolic pathway of meta parting of phenol showed that quality declaration can't clarify the decreased metabolic rates at inhibitory phenol concentrations. This phenomenon is due to in vivo inhibition of enzyme activities and notably to phenol hydroxylase activity. Moreover, other nonmetabolizable natural alcohols incited a comparative impact on bothspecific growth rate and phenol hydroxylase action, demonstrating that restraint was likely connected with changed layer smoothness, somewhat counterbalance by an adjustment in the unsaturated fat organization of cell lipids.

Hence we have seen from above literature review that various microorganism phenol degrading capacity has been tested and experimental results have been calculate on this basis. But all the above experiments the microorganisms are selected from the first and then their phenol degrading capacity is calculated. In our experiment we are testing the capacity of microorganism not from the contaminated sites such as hospital waste disposal area or refinery contaminated area but from the normal sites for example bus-station or cycle repair

store. To check whether they contain the phenol degrading microorganism or not and if yes, to which are those microorganism and to what extent they can degrade phenol.

# 3. PROBLEM DEFINITION AND PLAN OF WORK

Hence from the above study it is concluded that Phenol is a poisonous and dangerous substance still at low concentration and it is necessary to minimize the phenol concentration in wastewater to satisfactory levels. There are number of method accessible for handling of phenol, biological handling is particularly attractive as it has likely to approximately involve in the degradation of phenol entirely by producing harmless last yield and least derivative dissipate production. Also the methods used for phenol removal other than biodegradation are very costly and non-economic to be performed on large scale hence biodegradation of phenol is most economic process to degrade phenol from water. The only drawback of this process is that it is time consuming but if it works properly phenol can be removed from water most efficiently and economically. Bulk removal of phenol by solvent extraction, adsorption, chemical oxidation, incineration and other non-biological treatment methods suffer from serious drawbacks such as high cost and formation of hazardous by-products.

Also the study from the literature review that various microorganism's phenol degrading capacity has been tested and experimental results have been calculate on this basis. But all the above experiments the microorganisms are selected from the first and then their phenol degrading capacity is calculated. In our experiment we are testing the capacity of microorganism not from the contaminated sites such as hospital waste disposal area or refinery contaminated area but from the normal sites for example bus-station or cycle repair store. To check whether they contain the phenol degrading microorganism or not and if yes, to which are those microorganism and to what extent they can degrade phenol.

The work plan is explained in below steps:

- To find specific colonies degrading phenol by increasing the concentration of phenol.
- Degradation of phenol has to be measured and efficiency of different microorganism to degrade and time taken to reduce the concentration of phenol has to be measured.
- Effects of various parameter on phenol degradation:
    - pH
    - Temperature
    - *Initial concentration*
    - *Time*

# 4. MATERIALS AND METHODS

## 4.1 REQUIRED CHEMICALS:

The listed table below gives the various chemicals required for the experimental purpose:

| NAME OF CHEMICALS |
|---|
| Phenol |
| Ethyl alcohol |
| Surgical spirit |
| Nutrient broth |
| Agar |
| $KH_4PO_4$ |
| $K_2HPO_4$ |
| NaCl |
| $MgSO_4.7H_2O$ |
| $NH_4NO_3$ |
| $FeSO_4.7H_2O$ |
| $CaCl_2.2H_2O$ |
| $NH_4SO_4$ |
| 4-amino antipyrine |
| $K_3Fe(CN)_6$ |
| $NH_4OH$ |

Table 4.1 Chemicals required for experimental purpose

## *4.2 METHODS:*

There are several methods we have to undertake during experimental procedure for preparing pure cultures of microorganisms, preparing stock solution of phenol(10000ppm) and calculation, for degradation of phenol, pathways followed by microorganisms for degrading phenol, degradation after different inoculum age and different inoculum volume, degradation of phenol at different temperatures and $p_H$, preparation of buffer for adjusting $p_H$, 4-amino antipyrine method for detection of phenol by spectrophotometer and drawing calibration curve and calculating the phenol concentration.

### *4.2.1 PREPARATION OF PURE CULTURES OF MICROORGANISMS*:

Pure cultures are important to prepare because we store our microorganism in form of this pure cultures. Whatever the inoculum is needed for the degradation of the phenol is taken from these pure cultures. These pure cultures needed to be stored in very safe and dry place at constant temperature away from any impurities and contact of any other foreign microorganism. If it comes in contact of any foreign organism or in other words if the culture is disturbed by growth of any other microorganism in it, it will be very hard to know the impurity and the metabolism of actual microorganism will be disturbed hence careful handling of microorganisms pure cultures is needed after preparation of it. If the cultures get disturbed again the microorganisms needed to be isolated from the beginning.

The procedure/method for the preparation of pure culture (100ml) is:

- Measure 100ml distilled water with the help of measuring cylinder and take it in a 250 ml flat bottom conical flask.
- Measure 2g of nutrient broth which will be added in water for preparing the media for growth of microorganisms.
- Dissolve nutrient broth in water and sterilize it for about 15 minutes at a temperature of $121^oC$ in autoclave for removal of any contamination if present.
- Add 1ml inoculum of microorganism in flask after letting it cool down to room temperature in laminar flow hood.
- After 24 hours the pure culture will be ready for use.

### *4.2.2 PREPARING STOCK SOLUTION OF PHENOL (10000PPM):*

For preparing stock solution of any concentration we need to do have the solution of 99-100% concentration. Phenol chemical had been ordered and the phenol of 100% concentration had been present for preparation of the stock solution. Stock solution is very useful, for example if we need to prepare the solution of phenol concentration 100 ml it is very difficult to prepare it directly from the 100% concentrated phenol, as measure of that minute amount needs very costly calibrated pipettes but most importantly it needs a high degree of accuracy in handling chemical. Hence if stock solution of 10000 ppm or say 1000 ppm is prepare and kept the low concentration solutions can be prepared easily.

There is calculation involved in this procedure,

Let 'x' be the concentration of the stock solution needed to prepare in ppm. Let 'y' be the volume of the pure culture needed to prepare 100ml stock solution of concentration 'x'. Hence from balancing we know that for molar balance to be equal we have,

$$M_1V_1 = M_2V_2$$

$$X*100 = 10^6*y$$

$$Y = \frac{10^4}{x}$$

Hence we can make table for this calculation;

| **CONCENTRATION OF STOCK SOLUTION (Xppm)** | **AMOUNT OF CONCENTRATED SOLUTION(Yml)** |
|---|---|
| 1000 | 0.1 |
| 5000 | 0.5 |
| 10000 | 1.0 |
| 50000 | 5.0 |

Table 4.2 concentration of stock solution and amount of concentrated solution

### *4.2.3 DEGRADATION OF PHENOL:*

For degradation of phenol we need to prepare the minimal salt media. We can wary the initial concentration of phenol by the method specified in the above discussion. Minimal salt media is prepared instead of the nutrient broth so that all the nutrients that the organisms are getting from the nutrient broth for ex. carbon source or nitrogen source may be stopped. According to the phenol concentration of solution of 100 ml needed to prepare the amount of stock solution is taken and the rest distilled water is added to make over up to 100 ml. The minimal salt media provides the conditions of minimal slat required of the growth of the microorganisms. It provides the necessary nitrogen source and the metals in trace amount like calcium and iron. The minimal salt media composition per 100 ml solution is as follows:

| SALT | CONCENTRATION FOR 100 ML SOLUTION |
|---|---|
| $KH_4PO_4$ | 50mg |
| $K_2HPO_4$ | 150mg |
| NaCl | 50mg |
| $MgSO_4.7H_2O$ | 50mg |
| $NH_4NO_3$ | 100mg |
| $FeSO_4.7H_2O$ | 1mg |
| $CaCl_2.2H_2O$ | 1mg |
| $NH_4SO_4$ | 50mg |

Table 4.2.3 Minimal salt media composition

Procedure for inoculating minimal salt media with phenol concentration by the microorganisms:

- After preparing minimal salt media composition it has been sterilized in autoclave at 121$^{o}$C and 15 minutes so that all the contamination will be removed.
- Then the minimal salt medial is allowed to cool down to room temperature in laminar flow hood before inoculating it with microorganisms.
- Then media is inoculated by 1ml of pure culture of microorganism and then degradation of phenol starts and reading may be taken in different time intervals.

### *4.2.4 PATHWAY FOR PHENOL DEGRADATION:*

Phenol is being consumed by the microorganisms hence microorganisms have to follow certain mechanism. The critical step in the metabolism of aromatic compounds is the destruction of the resonance structure by hydroxylation and fission of the benzoid ring which is achieved by dioxygenase-catalysed reactions in the aerobic systems. Based on the substrate that is attacked by the ring cleaving enzyme dioxygenase, the aromatic metabolism can be grouped as ***Meta-pathway and Ortho-cleavage pathway*** these two pathways are specified for the degradation of phenol by microorganisms. Both the pathways are explained below with their reactions.

***META-PATHWAY FOR PHENOL DEGRADATION***:

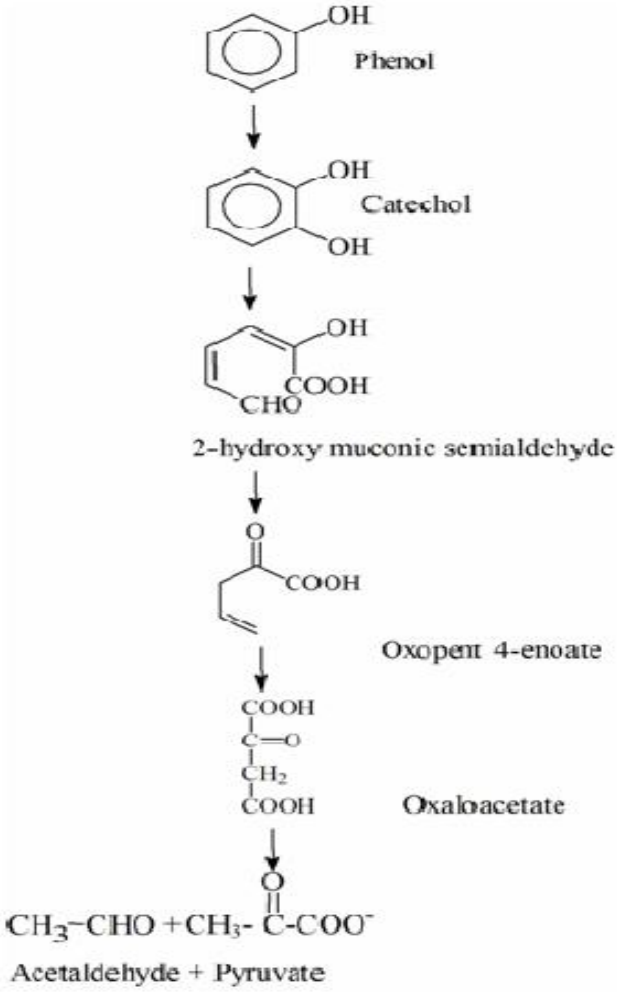


Fig 4.1 Meta pathway for phenol degradation

***ORTHO-PATHWAY FOR PHENOL DEGRADATION:***

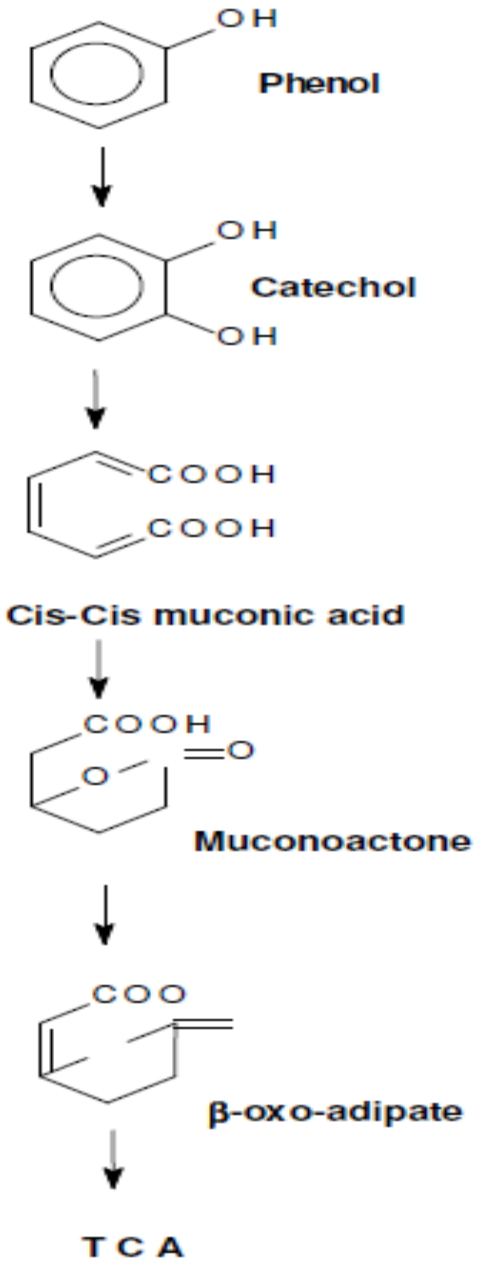


Fig 4.2 Ortho-pathway for phenol degradation

From both the above mechanism we come to know about the different pathways followed by which the microorganisms degrade phenol and their last products. In Meta pathway its acetaldehyde and pyruvate and in Ortho pathway its TCA (tri-carboxylic acid).

### *4.2.5 DEGRADATION BY DIFFERENT INOCULUM AGE AND DIFFERENT INOCULUM VOLUME:*

Up till now the inoculation was done with the inoculum of 1ml and age of 24 hours. But the degradation of phenol can be checked by inoculating the media by microorganisms of different inoculum age and different inoculum volume. The step for this experimental procedure is:

- Pure cultures of different microorganism were grown for the age of 24 hours, 48 hours and 72 hours.
- This different age inoculum was then taken to inoculate the minimal salt media with the phenol concentration.
- The result of the above experiment was noted in form of the absorbance by spectroscopic method.
- Also the different media composition was charged with the different inoculum volume.
- Five media sample were used and inoculated with five different volumes of inoculum mainly 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml.
- The results of the degradation were studied and amount of phenol degraded after particular time was measured by amount of phenol degraded per ml of inoculum.

### *4.2.6 DEGRADATION OF PHENOL AT DIFFERENT TEMPERATURES AND DIFFERENT $P_H$:*

Degradation of phenol was also studied at different temperatures mainly below $50^oC$ because all the naturally existing microorganisms in nature lose their growth at such a high temperature. Also for $p_H$ the different $p_H$ were used to detect the growth of microorganisms and the best one was detected with the maximum growth.

The results of this method were tabulated and for comparison were represented in form of graphs. It is easier to compare results when we have the graphical representation of the data materials.

### *4.2.7 PREPARATION OF BUFFER FOR ADJUSTING $P_H$ OF SOLUTION:*

For adjusting $p_H$ of media the buffer solution is used. Here we are using phosphate buffer for adjusting the value of $p_H$. Here the values for preparation of buffers are 104.5grams of $K_2HPO_4$ and 72.3grams of $KH_2PO_4$ dissolved in a litre of water the resulting $p_H$ for buffer is 6.8. This buffer is used for adjusting the $p_H$ of the medial by checking the $p_H$ and adding drop by drop wise solution of this buffer by micropipette.

### *4.2.8 4-AMINOANTIPYRINE METHOD:*

4-aminoantipyrine method is used for the detection of phenol concentration with the help of spectrophotometer. 4-aminoantipyrine method procedure is as below:

- The reaction mixture is prepared with the reactants necessary are 4-aminoantypirene solution prepared by dissolving 2grams of the chemical in 100ml of water (2% solution), potassium ferricynide solution prepared by dissolving 8 grams of potassium ferricynide in 100ml water (8%solution), also 2N ammonium hydroxide solution.
- The ammonium hydroxide solution calculations are as follows:
  The specific gravity of ammonium hydroxide is given to be 0.91. For 2N solution of ammonium hydroxide we should have 70.04 grams per litre. We need to prepare 100 ml solutions hence we require 7.004grams of ammonium hydroxide. For 7.004 gram of ammonium hydroxide we require 7.696ml (7.004/0.91) of concentrated ammonium hydroxide solution.
- For the spectroscopy method we require o.1ml of sample diluted 10 times with the 0.9 ml 0f water. Also we require our reaction agents as in amount of $25\mu L$ of 4-aminoantipyrine solution and potassium ferricynide solution and $50\ \mu L$ of 2N ammonium hydroxide solution. Set $p_H$ of reacting mixture at $7.9\pm0.1$.
- This all reacting mixtures (colouring mixtures) is again diluted with 9ml water and allowed to react for 15 minutes.
- After 15 minutes the absorbance of the colouring mixture is checked at 510nm using a spectrophotometer.

### *4.2.9 STRUCTURES AND REACTIONS FOR 4-AMINOANTYPIRENE METHOD:*

Structure and reaction of 4-aminoantipyrine with phenol is as follows:

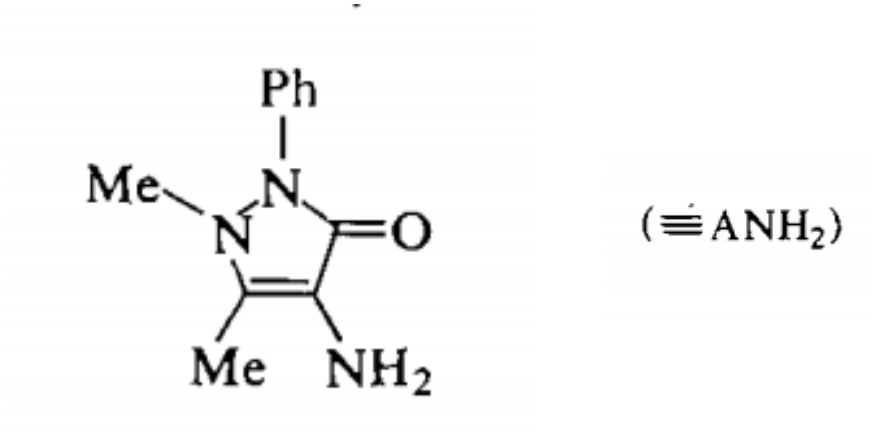


Fig 4.3 Structure of 4-aminoantipyrine

***Reaction:***

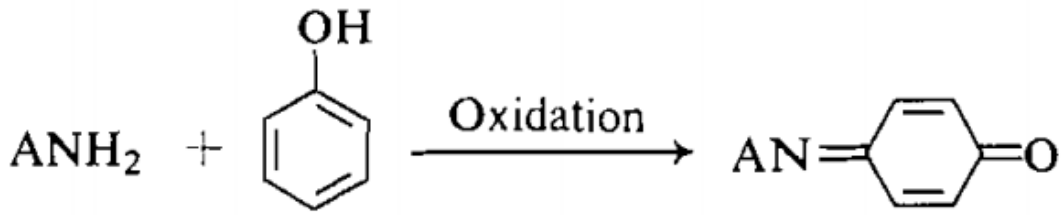


Fig 4.4 reaction of phenol and 4-amino-antipyrine

Quinone imide (Coloured compound mainly reddish brown in colour.)

Hence the 4-aminoantipyrine act as the colouring agent in presence of basic medium (here it is ammonium hydroxide solution) and this compound can be detected at the wavelength of 510nm.

### *4.2.10 PREPARATION OF CALIBRATION CURVE FOR PHENOL USING 4-AMINOANTIPYRINE METHOD:*

Calibration curve is prepared for determining the unknown concentration of phenol by the value of their absorbance. Calibration curve is one of the most important graphical representation of phenol concentration with respect to the absorbance from the spectrophotometer reading.

For preparation of calibration plot the steps are as follows:

- First prepare the 100 to 1000 ppm solutions of phenol with water at each 100ppm rise in concentration.
- Also prepare 10ppm to 100ppm concentration solution with water at each 1oppm rise in concentration.
- Now perform the 4-aminoantipyrine method on all the above 20 readings and note the result.
- Draw the graph of phenol concentration vs. absorbance where concentration being on the x-axis and absorbance being on y-axis.
- Now with the help of these two graphs we can calculate the concentration of phenol at unknown absorbance.

# 5. RESULTS AND DISCUSSION:

## 5.1 RESULTS:

Result for the above experiment includes the calibration values in form of tables and curves for determining the phenol concentration with respect to absorbance, the degradation of phenol by three microorganisms with respect to initial concentrations, temperatures, $p_H$, different inoculum age, different inoculum volume, % degradation with optimum temperature, $p_H$, inoculum age and inoculum volume. The graphs are also prepared for all the three microorganisms and the best one is found out.

### 5.1.1 CALIBRATION CURVE FOR CONCENTRATION VS. ABSORBANCE:

The values for calibration curve of the concentration of phenol from 100-1000ppm and its respective absorbance are as follows:

Table 5.1 Calibration curve values for concentration 100-1000ppm:

| Sl.no. | Phenol concentration(ppm) | Absorbance(510nm) |
|---|---|---|
| 1. | 100 | 0.211 |
| 2. | 200 | 0.271 |
| 3. | 300 | 0.349 |
| 4. | 400 | 0.401 |
| 5. | 500 | 0.450 |
| 6. | 600 | 0.500 |
| 7. | 700 | 0.574 |
| 8. | 800 | 0.651 |
| 9. | 900 | 0.679 |
| 10. | 1000 | 0.756 |

Calibration curve for phenol concentration 100-1000ppm:

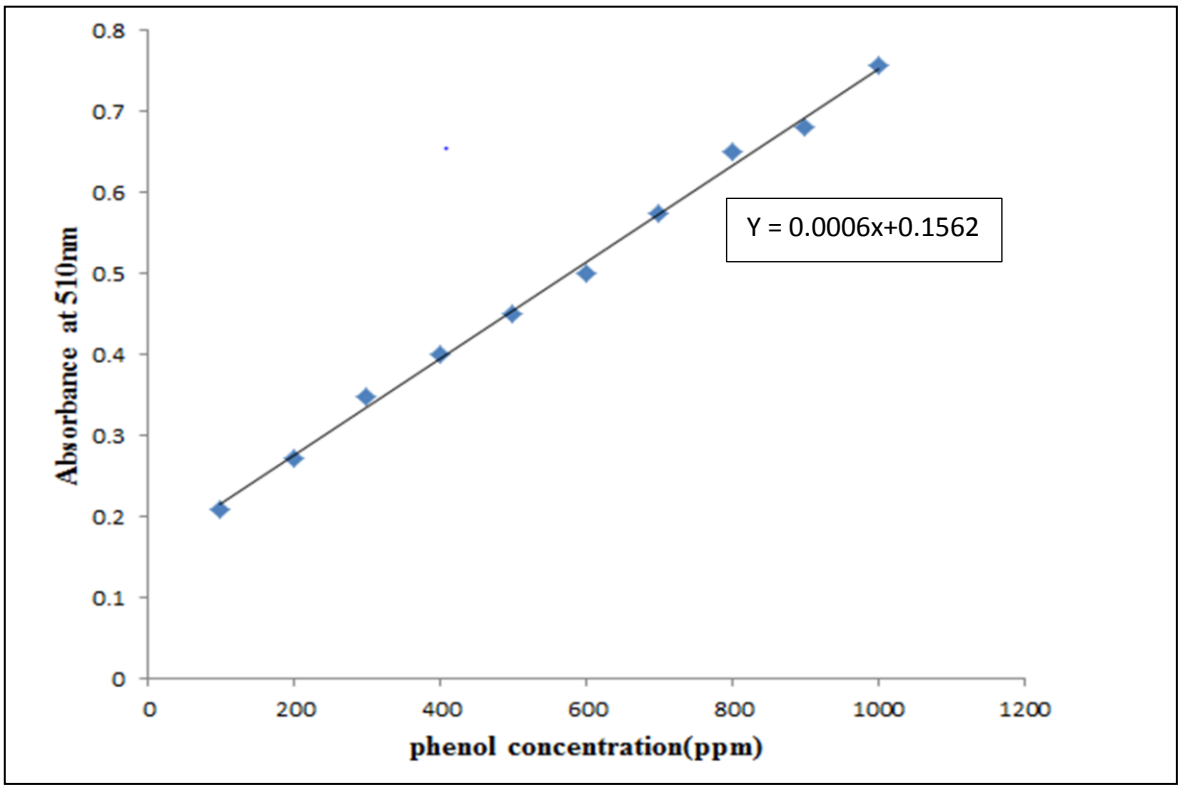


Fig 5.1 calibration curve for phenol concentration

The values for calibration curve for 10-100ppm:

| Sl.no. | Phenol concentration(ppm) | Absorbance(at 510nm) |
|---|---|---|
| 1. | 10 | 0.191 |
| 2. | 20 | 0.193 |
| 3. | 30 | 0.194 |
| 4. | 40 | 0.197 |
| 5. | 50 | 0.198 |
| 6. | 60 | 0.200 |
| 7. | 70 | 0.203 |
| 8. | 80 | 0.205 |
| 9. | 90 | 0.207 |
| 10. | 100 | 0.210 |

Table 5.2 Calibration curve values for concentration 10-100ppm

Calibration curve for phenol concentrations from 10-100ppm is:

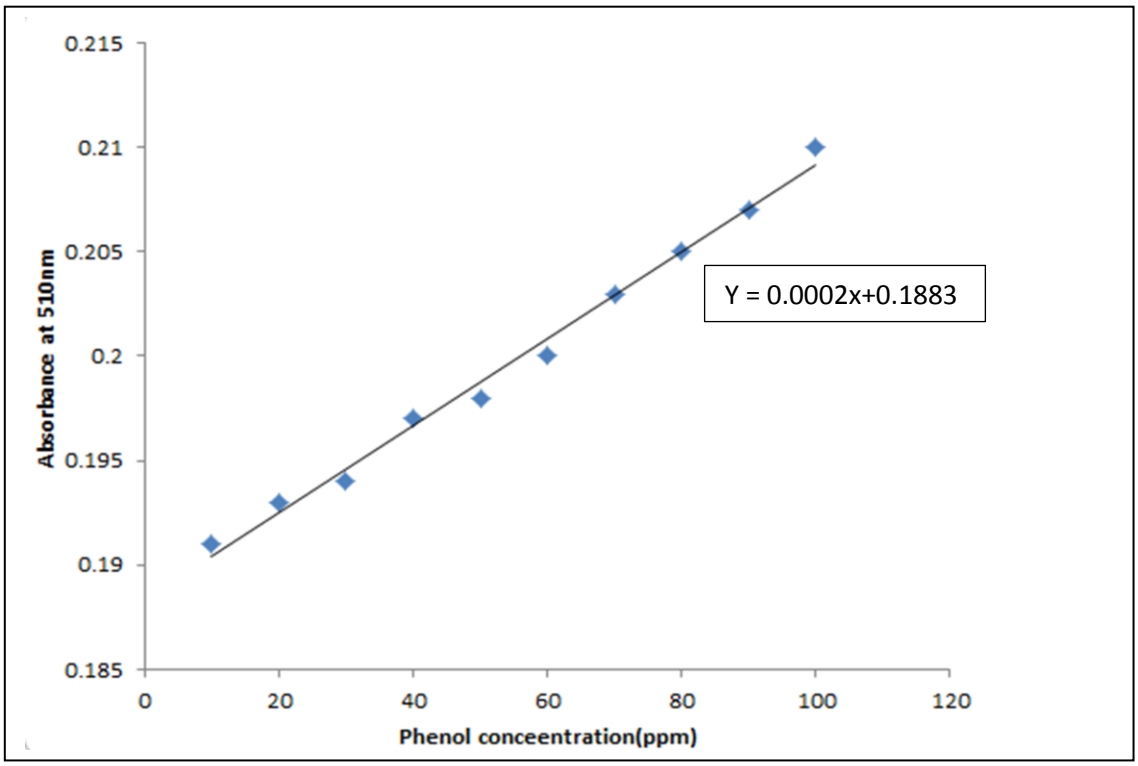


Fig 5.2 calibration curve for phenol concentration

### ***5.1.2 DEGRADATION STUDY OF PHENOL VS. INITIAL CONCENTRATION***:

Degradation study of phenol vs. initial concentration will give us the best operating condition or the best initial concentration for maximum percentage of degradation. The data for this analysis was as follows for all the three microorganisms. Microorganisms are named as $PR_1$, $PR_2$ and $PR_3$ respectively.

| **INITIAL CONC. (ppm)** | **$PR_1$ (absorbance)** | | | **$PR_2$ (absorbance)** | | | **$PR_3$ (absorbance)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24hr | 48hr | 72hr | 24hr | 48hr | 72hr | 24hr | 42hr | 72hr |
| 500ppm | 0.367 | 0.306 | 0.253 | 0.374 | 0.314 | 0.258 | 0.336 | 0.264 | 0.228 |
| 400ppm | 0.324 | 0.258 | 0.228 | 0.327 | 0.263 | 0.235 | 0.306 | 0.246 | 0.223 |
| 300ppm | 0.269 | 0.235 | 0.225 | 0.274 | 0.240 | 0.230 | 0.265 | 0.234 | 0.219 |
| 200ppm | 0.241 | 0.217 | 0.204 | 0.247 | 0.217 | 0.206 | 0.233 | 0.214 | 0.201 |
| 100ppm | 0.205 | 0.199 | 0.198 | 0.206 | 0.200 | 0.199 | 0.201 | 0.186 | 0.181 |

Table 5.2 (a). Tabulated form of absorbance data for various initial concentrations

The calculated values of concentration of phenol from the data obtained above from the spectrophotometer readings in form of absorbance using the calibration curve equation gives:

| **INITIAL CONC. (ppm)** | **$PR_1$(conc.ppm)** | | | **$PR_2$(conc.ppm)** | | | **$PR_3$(conc.ppm)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24hr | 48hr | 72hr | 24hr | 48hr | 72hr | 24hr | 42hr | 72hr |
| 500ppm | 352 | 251 | 162 | 363 | 263 | 170 | 306 | 180 | 123 |
| 400ppm | 278 | 170 | 123 | 285 | 178 | 131 | 252 | 154 | 112 |
| 300ppm | 189 | 132 | 114 | 196 | 139 | 122 | 180 | 132 | 105 |
| 200ppm | 141 | 102 | 80 | 152 | 102 | 88 | 131 | 96 | 77 |
| 100ppm | 82 | 57 | 51 | 89 | 61 | 57 | 73 | 51 | 42 |

Table 5.2 (b) Concentration data from absorbance data from calibration curve equation

Also this all data can be represented in form of graphs which will demonstrate better way of comparing this data with initial concentrations. The concentration curve are separately given for all the three microorganisms strain.

The graphical representation of above data is given below:

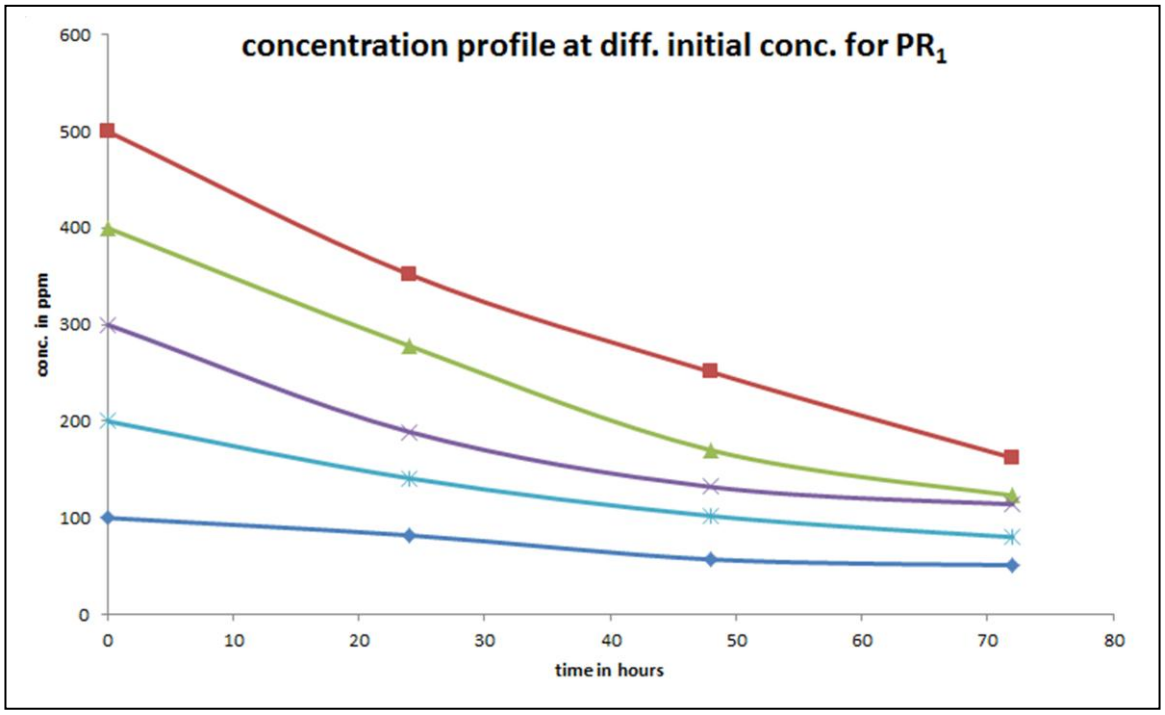


Fig 5.3 concentration profiles at different initial concentrations for $PR_1$

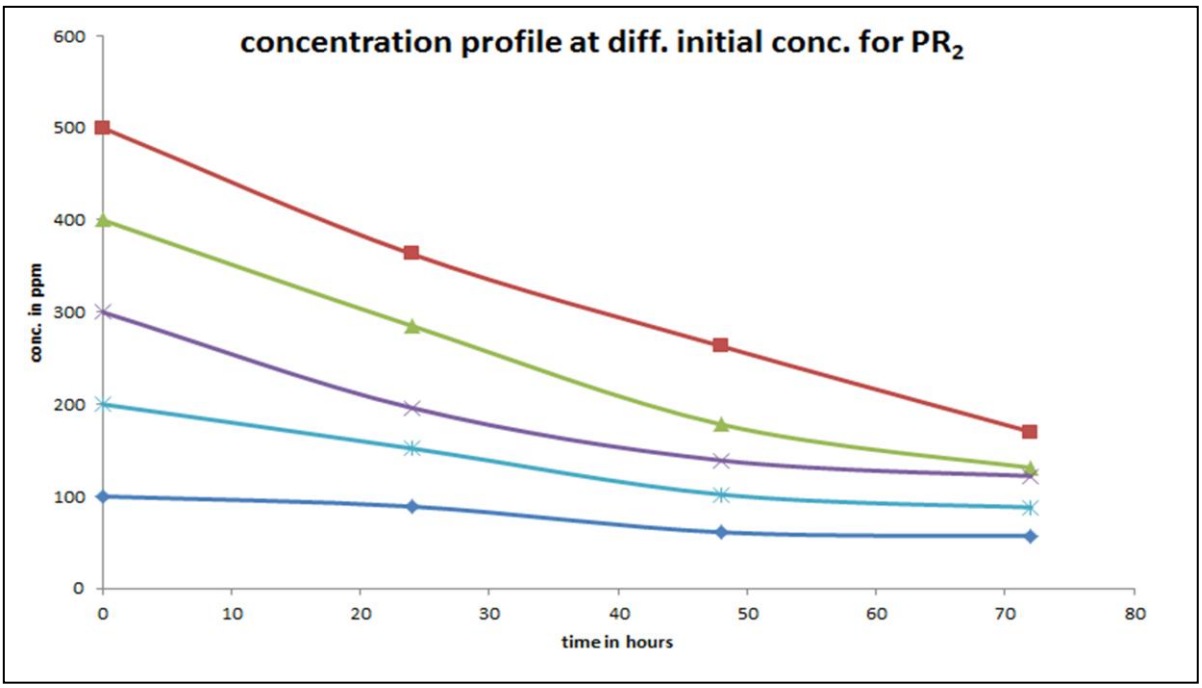


Fig 5.4 concentration profiles at different initial concentrations for $PR_2$

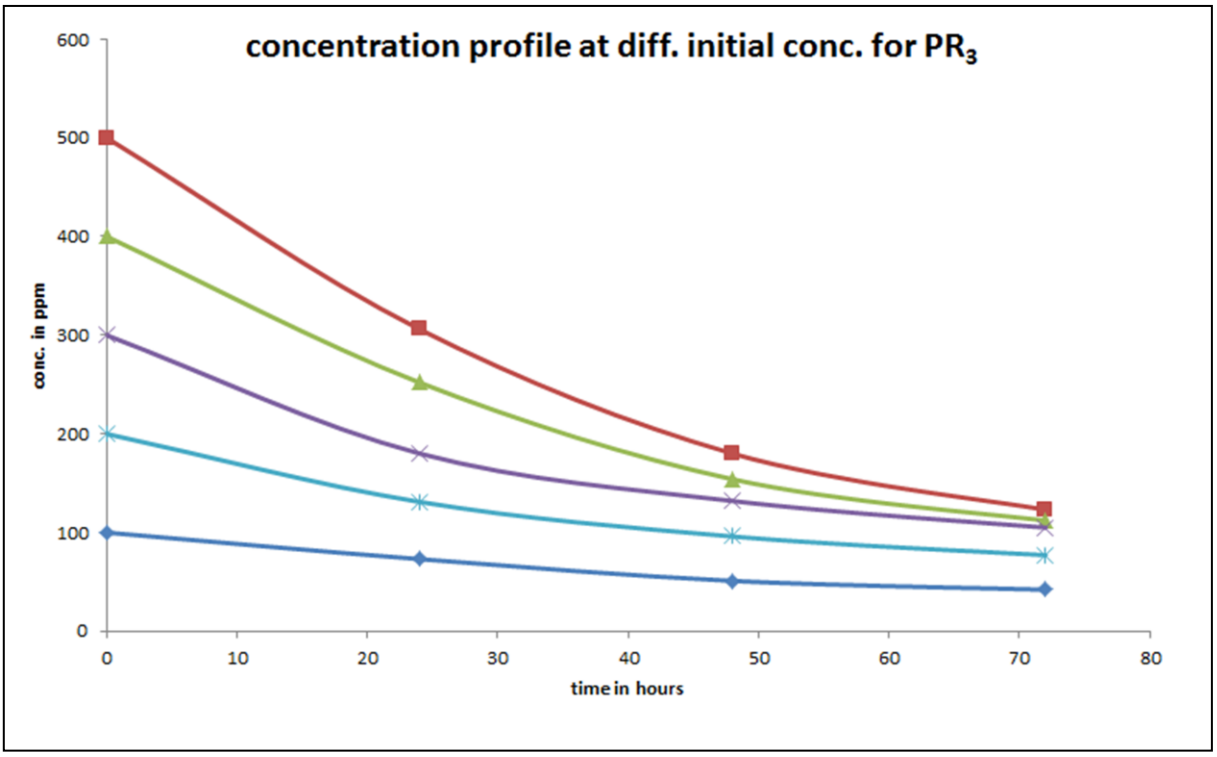


Fig 5.5 concentration profiles at different initial concentrations for $PR_3$

## *5.1.3 DEGRADATION STUDY OF PHENOL VS. TEMPERATURES*

In this experimental procedure we are checking the phenol degradation with respect to temperature variations. Three set ups are made certainly at 20, 30 and 40 degrees respectively and all three strains of microorganisms are tested for the degradation of phenol at different temperatures. Temperature is generally kept below 45$^{o}$C to avoid the resistance to growth of microorganisms as too coolness or hotness can lead to the increase in amount of dead biomass inside the media. Also initial concentration is taken to be 500ppm. The tabulated form is given below for the concentrations at different temperatures.

| **Microorganisms strains** | **Temperature ($^{o}$C)** | **Concentrations at different time interval** | | | |
|---|---|---|---|---|---|
| | | **0hr** | **24hrs** | **48hrs** | **72hrs** |
| $PR_1$ | 20 | 500 | 370 | 276 | 189 |
| | 30 | 500 | 352 | 251 | 162 |
| | 40 | 500 | 362 | 263 | 176 |
| $PR_2$ | 20 | 500 | 390 | 281 | 192 |
| | 30 | 500 | 361 | 264 | 168 |
| | 40 | 500 | 376 | 273 | 181 |
| $PR_3$ | 20 | 500 | 354 | 227 | 160 |
| | 30 | 500 | 304 | 184 | 127 |
| | 40 | 500 | 325 | 208 | 151 |

Table 5.3 Tabulated data of concentration for various operating temperatures

This data can also be represented in form of graphs separately for different strains:

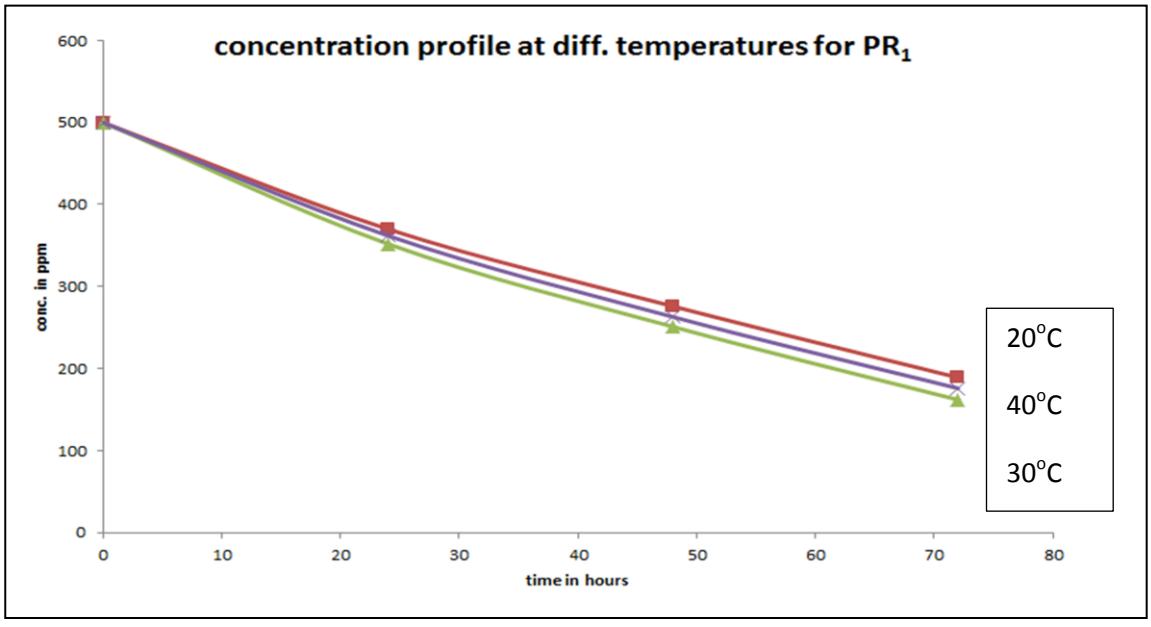


Fig 5.6 concentration profiles at different temperatures for $PR_1$

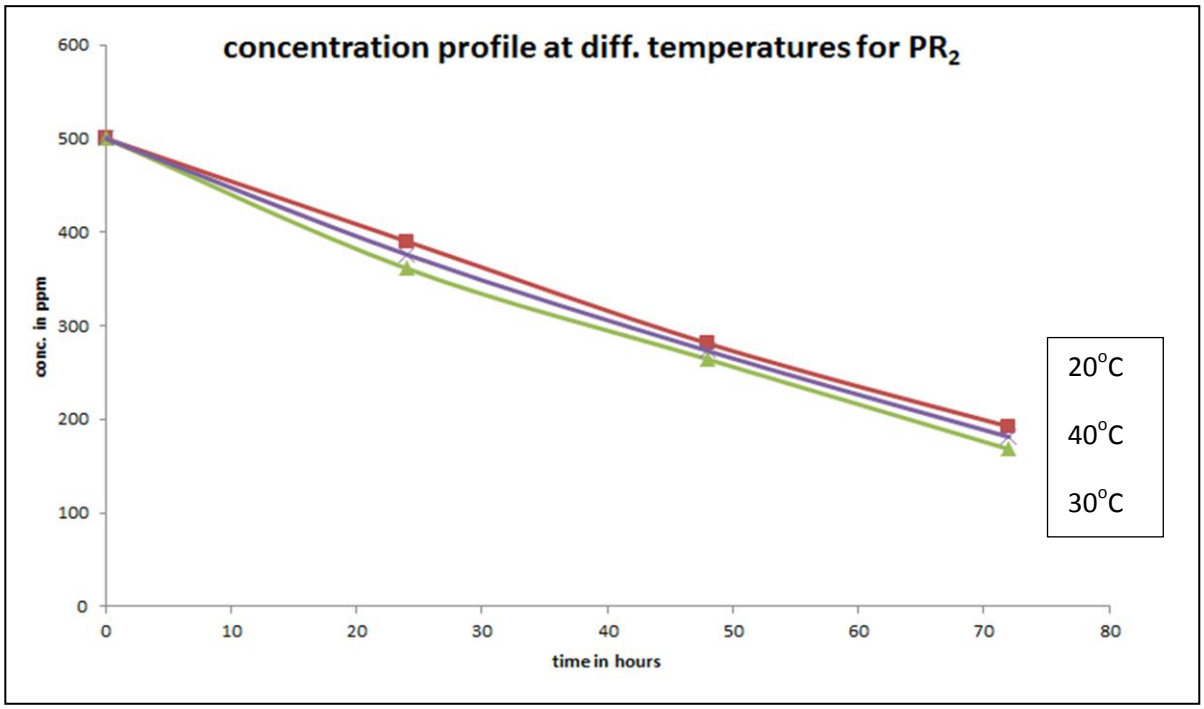


Fig 5.7 concentration profiles at different temperatures for $PR_2$

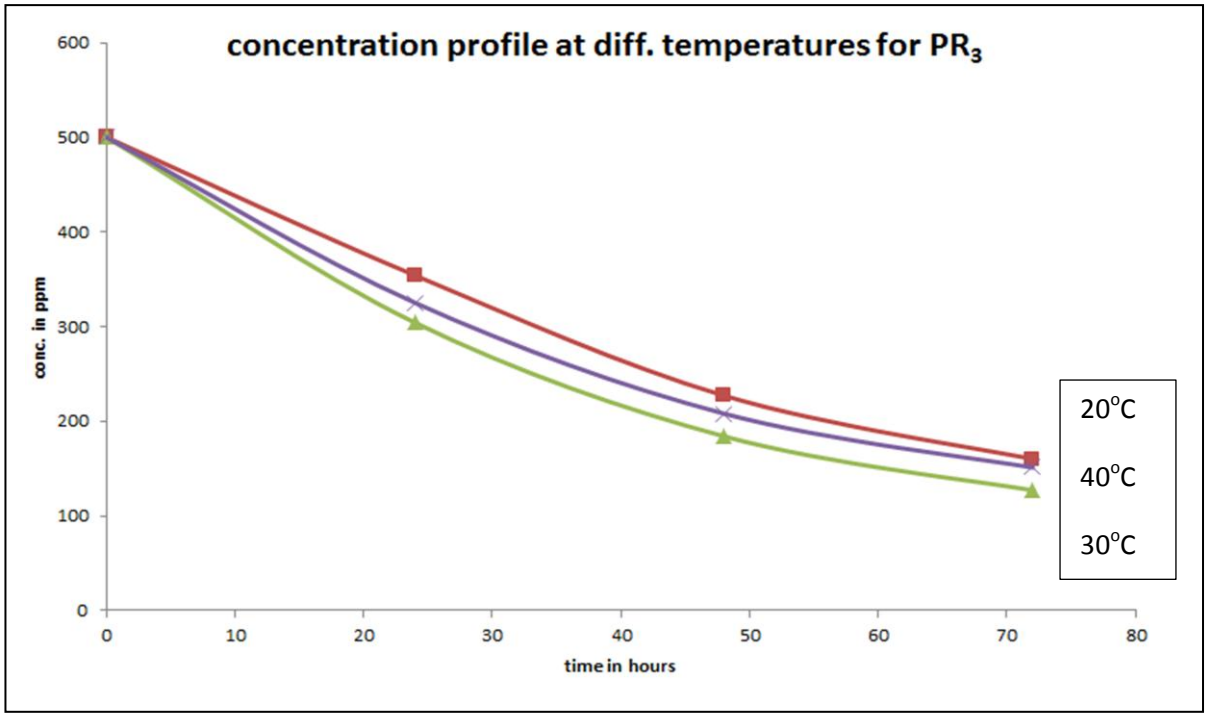


Fig 5.8 concentration profiles at different temperatures for $PR_3$

## *5.1.4 DEGRADATION STUDY OF PHENOL VS. $P_H$:*

In this experimental procedure we are checking the phenol degradation with respect to $P_H$ variations. Three set ups are made certainly of pH 5, 7 and 10 respectively and all three strains of microorganisms are tested for the degradation of phenol at different pH. pH is generally kept below 11 and above 4 to avoid the resistance to growth of microorganisms as too acidic and basic condition can hinder their growth. Also initial concentration is taken to be 500ppm. The tabulated form is given below for the concentrations at different pH.

| Microorganisms strains | pH | Concentrations at different time interval (ppm) | | | |
|---|---|---|---|---|---|
| | | 0hr | 24hrs | 48hrs | 72hrs |
| $PR_1$ | 5 | 500 | 389 | 276 | 181 |
| | 7 | 500 | 352 | 251 | 162 |
| | 10 | 500 | 370 | 260 | 169 |
| $PR_2$ | 5 | 500 | 393 | 293 | 187 |
| | 7 | 500 | 361 | 264 | 168 |
| | 10 | 500 | 382 | 279 | 181 |
| $PR_3$ | 5 | 500 | 345 | 220 | 158 |
| | 7 | 500 | 304 | 184 | 127 |
| | 10 | 500 | 327 | 203 | 145 |

Table 5.4 Tabulated data of concentrations for various operating pH of media

These can best be represented by graphs separately for each strains:

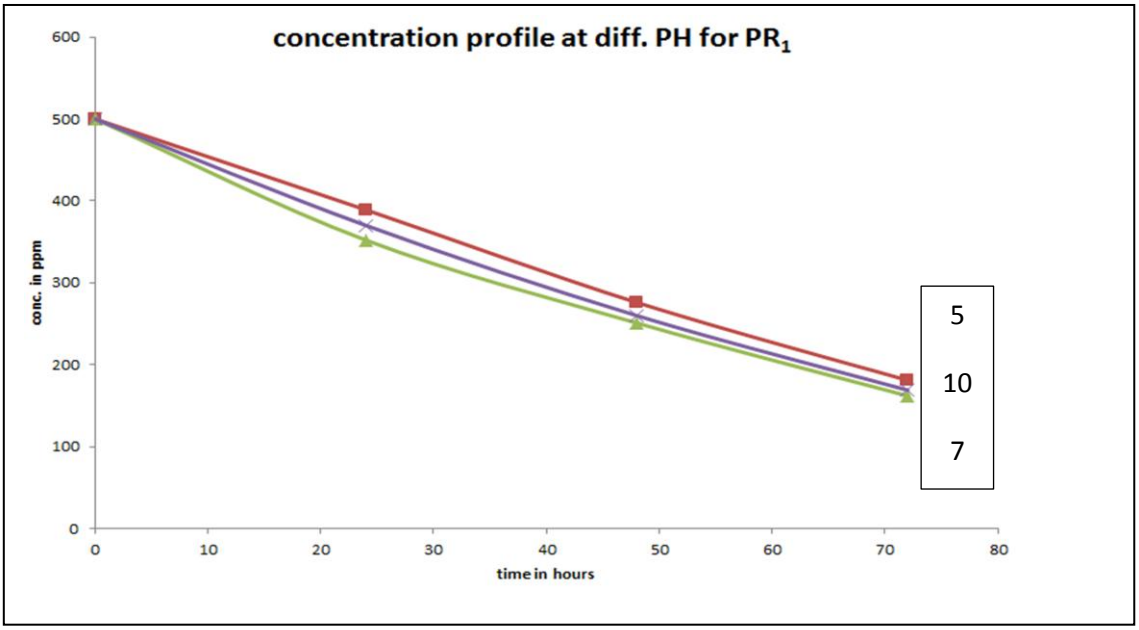


Fig 5.9 concentration profiles at pH for $PR_1$

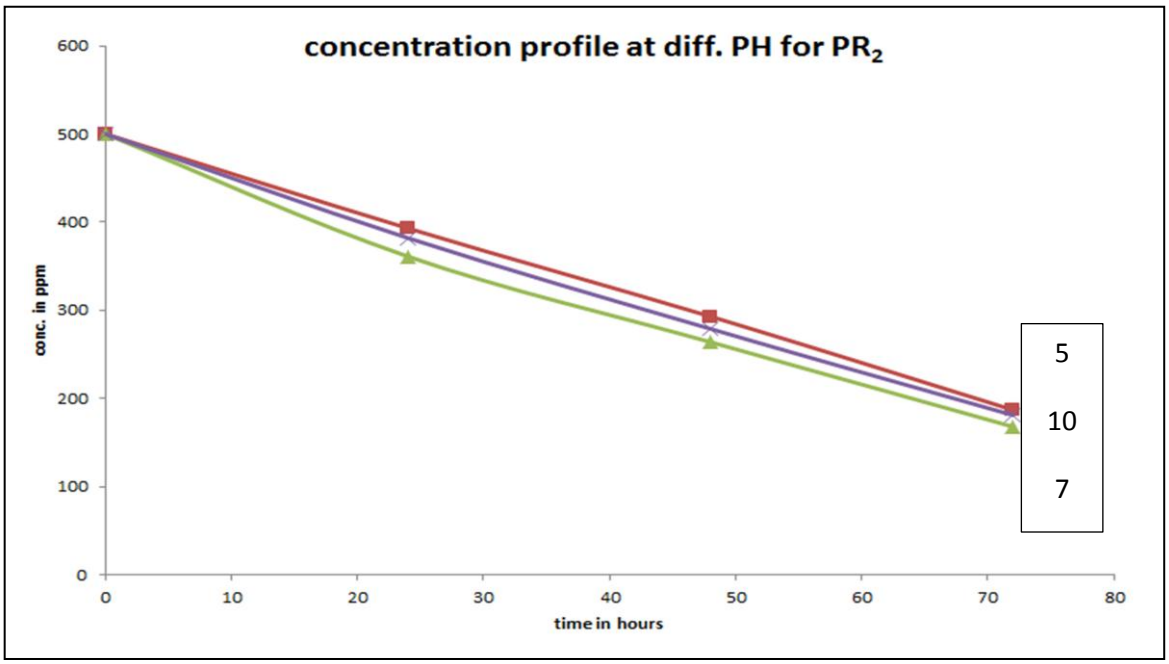


Fig 5.10 concentration profiles at pH for $PR_2$

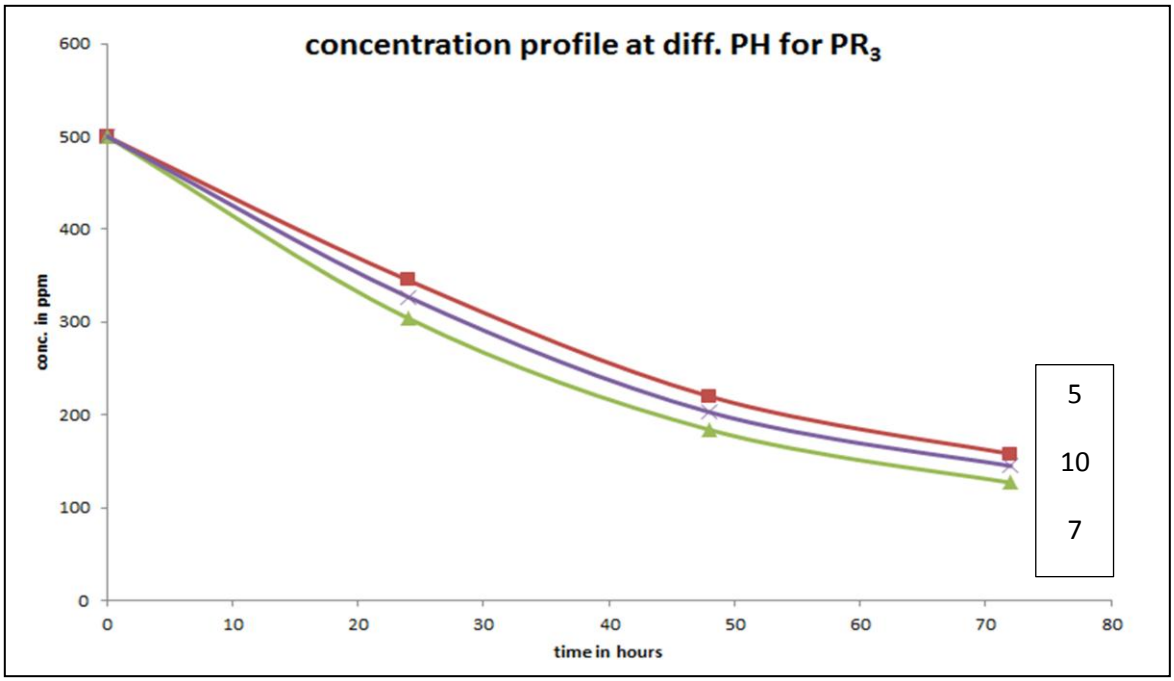


Fig 5.11 concentration profiles at pH for $PR_3$

## *5.1.5 DEGRADATION STUDY OF PHENOL VS. INNOCULUM AGE:*

Inoculum of different ages were used to inoculate the media was made study on the degradation pattern given by inoculum of different ages. The results of this experiment are tabulated below. The initial concentration being 500ppm and the temperature was maintained at 30$^{o}$C.

| Microorganisms strains | Age(hours) | Concentrations at different time interval (ppm) | | | |
|---|---|---|---|---|---|
| | | 0hr | 24hrs | 48hrs | 72hrs |
| $PR_1$ | 12 | 500 | 370 | 275 | 176 |
| | 24 | 500 | 352 | 251 | 162 |
| | 36 | 500 | 383 | 303 | 196 |
| $PR_2$ | 12 | 500 | 379 | 286 | 191 |
| | 24 | 500 | 361 | 264 | 168 |
| | 36 | 500 | 388 | 294 | 198 |
| $PR_3$ | 12 | 500 | 327 | 203 | 148 |
| | 24 | 500 | 304 | 184 | 127 |
| | 36 | 500 | 340 | 225 | 160 |

Table 5.5 Tabulated data of concentrations for different age of inoculum

This can also be represented in form of graph for better understanding separately for all strains:

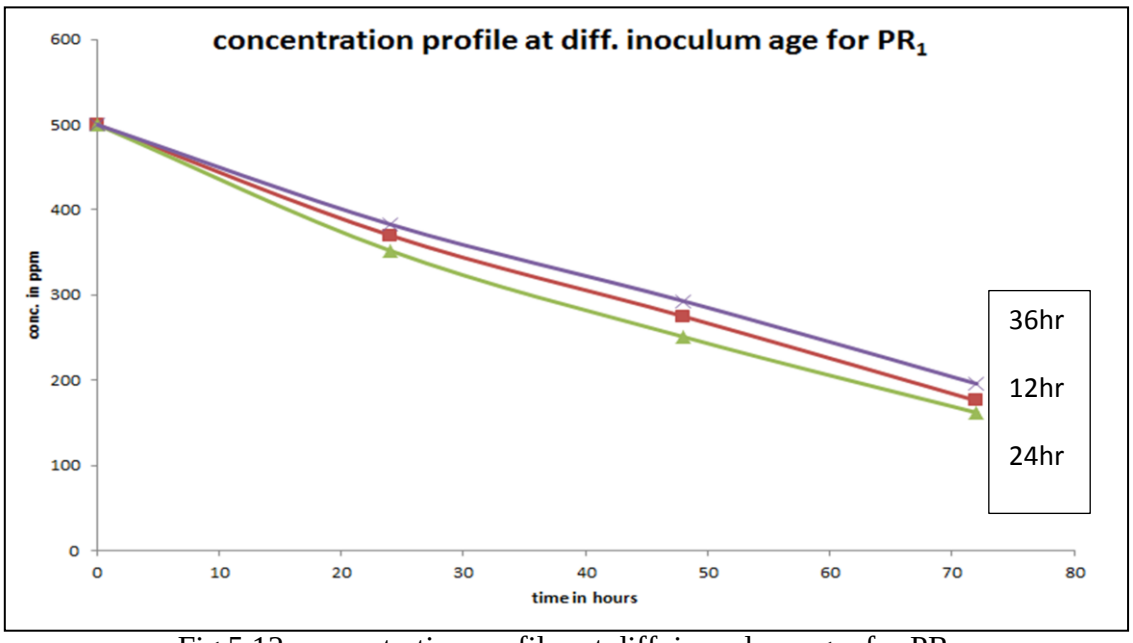


Fig 5.12 concentration profiles at diff. inoculum ages for $PR_1$

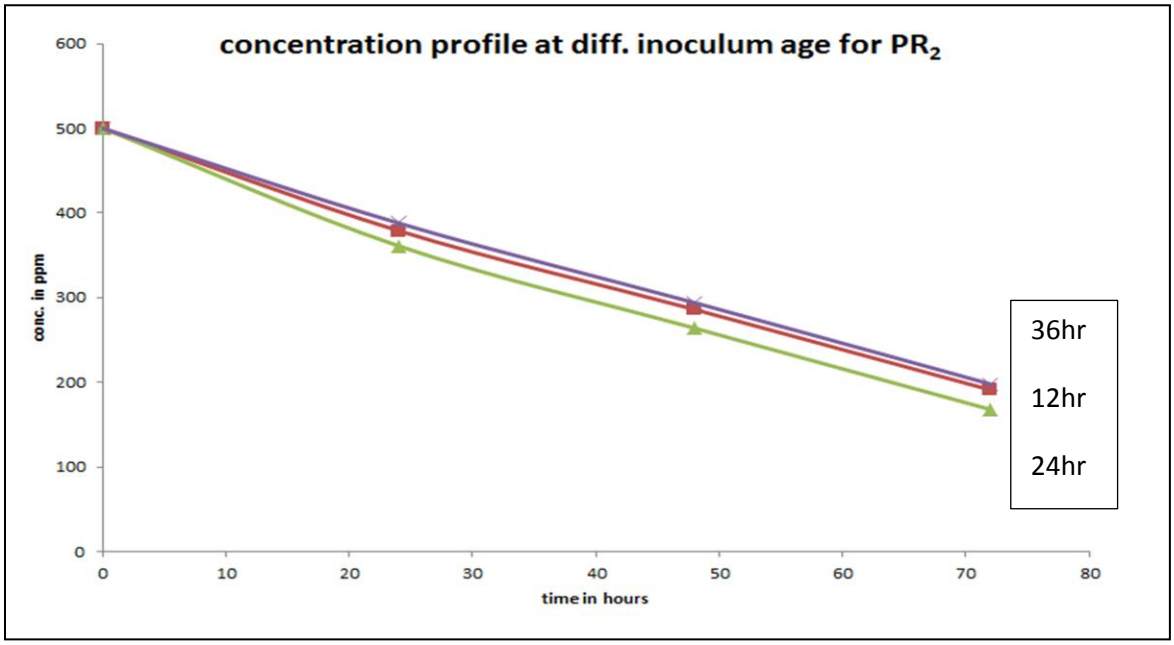


Fig 5.13 concentration profiles at diff. inoculum ages for $PR_2$

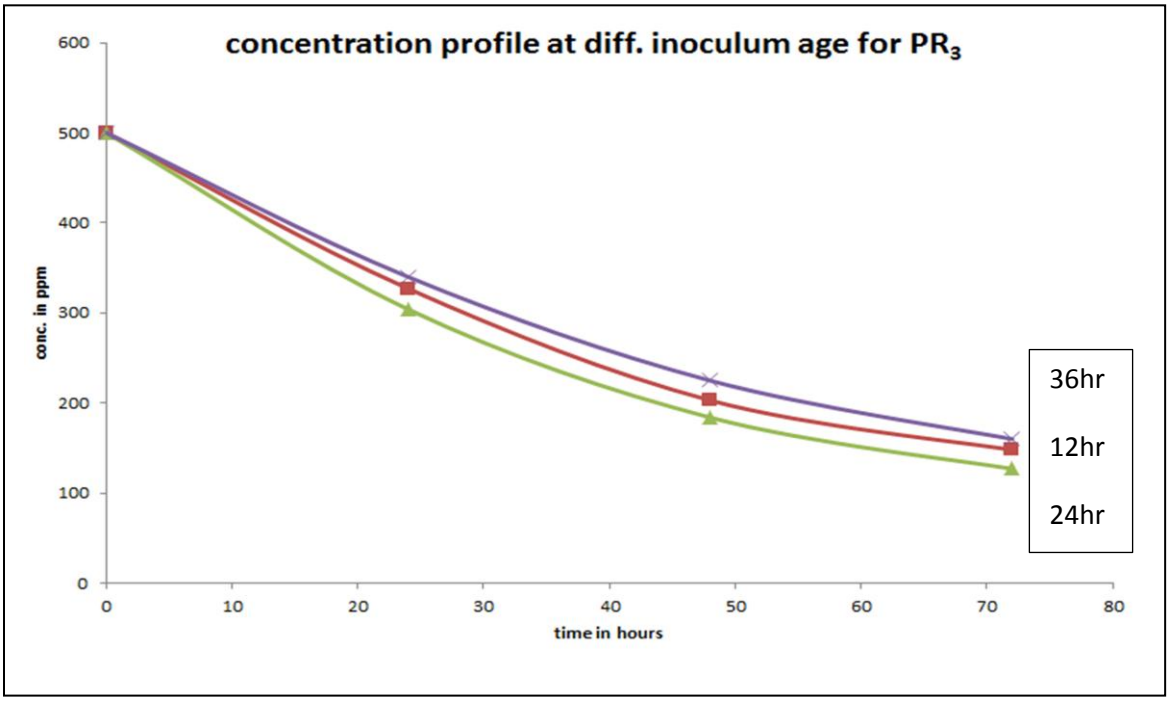


Fig 5.14 concentration profiles at diff. inoculum ages for $PR_3$

## *5.2 DISCUSSION:*

In all the above experiments we have seen the degradation of phenol by microorganisms. Discussing the result one by one, first for the initial concentration vs. phenol degradation, in this process we found out that the degradation of phenol rises with the rise in initial concentration of phenol. But there is no significant difference of degradation between 400ppm and 500ppm of phenol. Initial concentration of 500 ppm gives 76% of degradation in 72 hours and 400ppm gives 73% degradation. Hence we recommend 500ppm should be the optimum initial concentration of phenol. There must be higher efficiencies at higher concentration levels but with increase in concentration of phenol above this limit do not show significant change maximum efficiency is 76.8% for 800 ppm phenol there is no change of rate of degradation above this limit.

Phenol degradation also gets effected by the change in temperature. Degradation gets effected by change in temperature because the growth of microorganisms depends on temperature also. Temperature plays an important role in degradation of phenol and optimum temperature needed to be found out. All three microorganisms when tested under different temperatures showed maximum degradation at room temperature that is $30^{o}C$. Temperature fluctuations above and below $30^{o}C$ leads to decline in rate of degradation of phenol. The maximum degradation is shown by the strain $PR_3$ at room temperature nearly 75.8% degradation has been obtained in 72 hours.

Also the pH of media plays a vital role in the growth of microorganisms every microorganism does not sustain similar type of environment some shows higher growth in basic media but some shows higher growth in acidic. Generally organisms that are found in the surrounding under standard atmospheric condition tend to show maximum growth in neutral medium. Here while comparing three strains against pH all the strains showed the maximum degradation in neutral media with pH 7. If the pH either increased or decreased the degradation rate of microorganism reduced. Hence for these microorganism strains the optimum pH is 7.

When the media with initial concentration 500ppm was inoculated with the inoculum of different ages the degradation was effected by it. Three different ages of inoculum was utilized mainly of age 12 hours, 24 hours and 36 hours. From these three strains the inoculum of age 24 hours showed the maximum degradation because it is the time when these microorganisms showed their peak growth. Then next highest was the degradation from

inoculum age of 12 hours. 36 hours inoculum showed the least degradation since this is the age when microorganisms enter the death phase of their life cycle. As such there no big difference in the degradation rate but for efficient process inoculum of 24 hours age should be used.

We also compared the degradation results by inoculating media with different inoculum volume. But the degradation result does not gets effected by the inoculum volume degradation per ml of inoculum remains same for the room conditions.

## ***6. CONCLUSION:***

From the above results and discussions we came to know about the different properties affecting the degradation of phenol. From all the study made it is clear that the strain $PR_3$ is showing the maximum degradation in all conditions. While comparing the initial concentrations also this strain showed the maximum degradation of around 76%. Hence this strain or this microorganism is chosen to be the best one. It gives maximum degradation in pH =7 and at temperature of $30^oC$, with inoculum age to be around 20-24 hours. To know the specific properties of this strain characterization of microorganism has to be done. At laboratory level only some tests can be done but for actual name and species of microorganism it has to be sent to chemical institutes which can give the name of microorganism by performing all the characterization tests on it.

Microorganism was send to *NCIL Pune,* and after characterization report the name of microorganism was found out to be *staphylococcus lentus.* This is the family of bacteria found in contaminated places like car garages, vehicle wash centres, cycle repair stand etc.

Hence our study was completed here and we found out the best microorganism degrading phenol and its optimum conditions.

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